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High-affinity binding sites for VIP in renal cortical membranes: Possible role of VIP in renal transport

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High-affinity binding sites for VIP in renal cortical membranes: Possible role of VIP in renal transport. We studied binding and degradation of vasoactive intestinal peptide (VIP) by highly purified brush border and basolateral membranes from rabbit kidney cortex. Brush border and basolateral membranes were capable of 73 and 49% degradation of VIP after 20 minutes, and the degradation was totally prevented by bacitracin. There was 66 and 87% specific binding of ^{125}I-VIP to brush border and basolateral membranes, respectively. ^{125}I-VIP binding to renal membrane was displaced in a dose dependent fashion by unlabeled VIP with half maximal displacement at 2×10^{-7} M. Other related peptides failed to displace VIP. Scatchard analysis showed one single class of receptors for VIP in both membranes with similar Kd (0.5×10^{-7} M) but higher number of binding sites (B_{max}) in the basolateral membranes than in the brush border membranes (22.0 vs. 4.4 pmol/mg protein), respectively. Forty-eight percent of VIP binding to brush border membranes could be explained by cross contamination of these membranes with basolateral membranes. We examined the effect of VIP on Na-H antiporter, Na-dependent glucose uptake and Na-dependent phosphate uptake by isolated proximal tubule suspension. In acid loaded proximal tubules VIP (10^{-6} M) inhibited total and amiloride-sensitive ^{22}Na uptake by 35 and 75%, respectively, as compared to control. On the other hand VIP failed to inhibit Na-dependent methyl α-^{14}C-glucopyranoside and Na-dependent ^{32}phosphate uptake. VIP failed to stimulate cyclic AMP generation by proximal tubule suspension while PTH showed the expected stimulation. Our results demonstrate the presence of specific binding for VIP in highly purified cortical membranes and suggest an effect of VIP to inhibit the Na-H antiporter by a mechanism independent of cyclic AMP.

The role and mode of action of vasoactive intestinal peptide (VIP) in renal function is largely unknown. This "gastrointestinal peptide" neurotransmitter is widely distributed and has diverse effects in a variety of tissues [1–4]. Immunoreactive VIP has been localized to renal neurons, mainly in the cortex, associated with the renal artery and arterioles [5, 6]. Because of the known potent vasodilator properties of VIP in other tissues and the proximity of immunoreactive staining to arteries and arterioles, it has been hypothesized that VIP may have a renal vasodilator role. Stimulation of water and electrolyte secretion in the intestine by VIP has also been well characterized, and a role for VIP regulation of renal tubular transport has been suggested [7–13]. Moreover, there is strong evidence that VIP has a direct effect on electrolyte transport by epithelia which have strong analogy to segments of the nephron [14–17].

Calam et al [18] demonstrated that intravenous infusions of VIP into healthy men resulted in a reduction in renal vascular resistance in the face of reduced systemic blood pressure. Rather than stimulating excretion, a net increase in fractional reabsorption of sodium, potassium and chloride was observed, which was attributed to a hemodynamic effect of VIP. In contrast, Dimaline, Peart and Unwin [19] demonstrated that intravenous VIP infusion into conscious rabbits resulted in significant enhancement of sodium, potassium and chloride excretion despite a fall in renal blood flow and glomerular filtration rate. When VIP was infused directly into the renal artery of anesthetized dogs, renal vasodilation was demonstrated [20]. In the isolated perfused kidney, however, VIP infusion had no effect on vascular resistance but produced significant increases in urine volume and the fractional excretion of electrolytes, strongly suggesting an action of VIP directly on tubular transport [21].

Thus, the results from in vivo studies of the effect of VIP in renal function has been contradictory, some studies supporting a primary hemodynamic action and other studies a primary effect on tubular transport [18, 19]. A clear understanding of the mechanism of VIP regulation of kidney function is not possible in these in vivo studies because a multitude of uncontrollable systemic factors can alter the renal response to intravenous VIP. In the present study, we studied binding and degradation of VIP by highly purified luminal and basolateral membranes and evaluated the effect of VIP on tubular transport by isolated proximal tubule suspension of the rabbit kidney.

Methods

Experiments were performed on New Zealand white rabbits (weighing 3 to 6 lb) which were fed a normal diet. Preparation of brush border and basolateral membranes of renal cortex were performed utilizing the technique of Kinsella et al [22] as previously described by our laboratory [23, 24]. Brush border membranes were enriched tenfold in alkaline phosphatase and γ-glutamyl transferase and basolateral membranes were enriched 13-fold in Na-K-ATPase activity. Cross contamination of basolateral (BL) with brush border (BB) membranes vesicles and vice versa were calculated using the absolute activities of alkaline phosphatase and Na-K-ATPase activities in the prep-

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arations according to the equation described by Cheng and Hammerman [25]. The equation for cross contamination of BBMV with BLMV was as follows:

$$\frac{\text{BBMV alkaline phosphatase activity} \times 100\%}{100\% - \%\text{ contamination of BLMV in BBMV}} =$$

$$\frac{\text{BBMV alkaline phosphatase activity} \times 100\%}{\%\text{ contamination of BLMV in BBMV}}$$

A similar formula was used to calculate cross contamination of BLMV with BBMV.

Binding and degradation of ^{125}I-VIP by renal cortical membranes

For binding and degradation experiments we utilized ^{125}I-VIP (20 pM) which was added to a suspension of basolateral or brush border membranes (100 to 300 μg protein) in specially coated tubes contained a buffer of the following composition: mannitol 100 mM, glycylgcine 25 mM, bovine serum albumin 1%, pH 8 at room temperature [26]. Adsorption of ^{125}I-VIP by the tubes was prevented by precoating the tubes with polyethylenimine and an organosilane. For the degradation experiments, membranes were incubated in the absence and in the presence of 1% bacitracin or other protease inhibitors for 10 to 30 minutes [26, 27]. The reaction was terminated by adding 250 μl of 20% TCA. Separation of the membranes from the incubation medium was accomplished by centrifugation at 4°C at 8000 g for 10 minutes. The radioactivity in pellet and in the supernatant was determined and the amount remaining in the supernatant was considered degraded ^{125}I-VIP. Once we determined that bacitracin was an effective inhibitor of the degradation of VIP, all subsequent experiments were performed in the presence of 1% bacitracin [27]. For binding experiments the membranes were incubated in the above described buffer in the presence of 20 pM ^{125}I-VIP for 20 minutes at 22°C and in the absence and presence of varying amounts of excess unlabeled VIP. The reaction was terminated by addition of ice-cold buffer containing 5% bacitracin followed by rapid filtration through specially coated filters. The filters were rinsed twice, removed, dried overnight and counted in a gamma counter. Nonspecific binding was determined by the amount of ^{125}I-VIP bound to the membrane in presence of excess unlabeled VIP.

Isolation of proximal tubule suspension

For experiments examining the effect of VIP on tubular transport we utilized a proximal tubule suspension isolated according to the method of Chung et al [28]. In brief, New Zealand white rabbits (3 to 6 lb) were anesthetized with nembutal (50 mg/kg). The aorta was cannulated and the kidneys perfused in situ with sterile serum-free medium (DMEM/F12) containing particulate iron oxide. The kidneys were dissected free from the abdominal cavity, the capsule was removed, and the cortex was then dissected free from medullary tissue and homogenized in a Dounce homogenizer. The homogenate was serially filtered through two nylon mesh screens having pore sizes of 250 microns and 44 microns. The material which traversed the former screen but was retained in the latter screen contained both tubules and glomeruli—the latter being removed with a sterile magnet. The resultant tubule suspension was briefly exposed to collagenase, washed with sterile medium and used for transport by suspending the tubule suspension in the appropriate buffer. Electron microscopy studies of the isolated tubules showed that more than 98% were proximal tubules.

^{22}Na uptake by proximal tubule suspension in presence of a pH gradient

^{22}Na uptake by isolated proximal tubules was performed as follows: the tubule suspension was washed and pre-equilibrated for 20 minutes in a buffer containing 120 mM TMA-Cl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 18 mM MES, 14 mM Hepes, 14 mM Tris-Base, pH 6.4. The tubules were resuspended in this incubation buffer containing 1% bacitracin and with 10^{-5} M VIP or equal volume of the vehicle (acetic acid), and incubated for 30 minutes at 37°C. Transport buffer contained 119 mM TMA-Cl, 25 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 28 mM Hepes, 18 mM Tris-Base, pH 7.4. Ouabain at 0.1 mM was included to inhibit Na-K-ATPase [29]. Using a ratio of 80 μl transport buffer to 10^6 cells in 20 μl, the final extracellular pH was 7.4 with intracellular pH of approximately 6.4. (In other experiments utilizing the fluorescent probe BCECF in isolated proximal tubular suspension, we showed that intracellular pH approaches the extracellular pH with 20 minutes of incubation.) This low intracellular pH provides an outward H^+ gradient which favors Na uptake. In addition, 1.0 μCi ^{22}Na was added to the transport buffer. Amiloride at 1 mM was used to inhibit the Na uptake. The time course was one minute. This time was chosen because preliminary experiments indicated that the rate of ^{22}Na uptake was linear up to two minutes. The reaction was stopped by addition of 1 ml of ice-cold 149 mM LiCl, 0.5 mM Tris base, 0.5 mM Hepes, pH 7.5 and filtered rapidly through a Millipore filter with an additional 15 ml of stop-solution. The filters with trapped cells were assayed for radioactivity in a scintillation counter. The amiloride inhibitable Na uptake was expressed as Na-H antiporter activity in pmol/mg protein.

Na-dependent ^{32}phosphate uptake

Na-dependent ^{32}phosphate uptake by isolated proximal tubule suspension was measured in the presence and in the absence of sodium as described by Paujeol and Vandewalle [30]. The tubule suspension was incubated in a buffer containing 280 mM mannitol, 10 mM Hepes, 2 mM glutamine, 1 mM pyruvate, 0.1 mM $MgSO_4$, 0.25 mM $CaCl_2$, pH 7.35. The tubules were resuspended in this incubation buffer containing 1% bacitracin and with 10^{-5} M VIP or equal volume of the vehicle (acetic acid), and incubated for 30 minutes at 37°C. The transport buffer contained 140 mM NaCl or KCl, 10 mM Hepes, 4 mM potassium phosphate with 10 μCi, ^{32}P at 37°C. To initiate the uptake, 10 μl of tubule suspension was added to the transport buffer for 1 to 60 minutes, and after this time interval the reaction was stopped by adding one ml of ice-cold solution containing 149 mM LiCl, 0.5 mM Tris-Base and 0.5 mM Hepes. The tubule suspension was centrifuged, washed and the pellet was dissolved in NaOH, and radioactivity was determined. ^{32}Phosphate uptake experiments were performed in the presence and in the absence of 10^{-5} M VIP in the presence and in the absence of NaCl (NaCl was replaced by KCl).

Na-dependent methyl α-D ^{14}C-glucopyranoside by isolated proximal tubule suspension

Tubule suspension was washed twice with a preincubation buffer containing 250 mM mannitol, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 14 mM Hepes and 10 mM Tris-Base to remove any remaining DMEM-F12 solution in the tubules [28]. The tubules were resuspended in this incubation buffer containing 1% bacitracin and with 10^{-5} M VIP or equal volume of the vehicle (acetic acid) and incubated for 30 minutes at 37°C. After preincubation of the tubules, they were transferred to precoated polystyrene tubes and incubated in 125 mM NaCl or KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 28 mM Hepes, 18 mM Tris-Base, 1 mM methyl (α-D-^{14}C-glucopyranoside (α MGP) and 50 μM ^{14}C-α-MGP for 1 to 60 minutes at 37°C. The reaction was then stopped by adding 4 ml ice-cold solution containing 149 mM LiCl, 0.5 mM Tris-Base, 0.5 mM Hepes pH 7.50 and rinsed twice on Millipore GFA filters. The filters containing the tubules were then counted in a beta scintillation counter.

Cyclic AMP measurement

Cyclic AMP was measured in isolated proximal tubule suspension before (basal cyclic AMP generation) and after stimulation with PTH, forskolin, vasopressin or VIP. Isolated tubule suspension was incubated for various times in the presence and absence of these known simulators of adenylate cyclase and VIP with or without the addition of the phosphodiesterase inhibitor, 1-isobutyl-3-methylxanthine (IBMX), and in the presence of inhibitors of VIP degradation [31, 32]. The reaction was stopped by addition of ice-cold buffer (DMEM/F12) and rapid centrifugation of suspended cells in a Beckman microcentrifuge. Cells were resuspended in buffer (potassium phosphate 5 mM, KCl 150 mM, EDTA 2 mM) containing IBMX (0.5 mM), sonicated 10 seconds, boiled for six minutes and then centrifuged. Cyclic AMP was measured in the supernatant by a radioimmunoassay using antibody obtained from ICN Immunologicals, Inc.

Results are presented as mean ± SEM. The *t*-test for paired or unpaired data was used wherever appropriate. Linear regression was calculated by the least square method.

Results

Degradation of ^{125}I-VIP by brush-border basolateral ^{125}I-VIP renal membrane

Figure 1 shows the percent degradation of ^{125}I-VIP by highly purified brush border and basolateral membranes. It can be seen that brush border membranes rapidly degraded ^{125}I-VIP with a rate of 60% degradation at 10 minutes, and this rate of degradation remained stable thereafter. In contrast, degradation by basolateral membranes was 20% at 10 minutes and increased to 55% at 30 minutes. Figure 2 summarizes the degradation of ^{125}I-VIP at 20 minutes by brush border and basolateral membrane in the absence (open bars) and in the presence of 1% bacitracin (shaded bars). It is clear that bacitracin totally prevented the degradation of VIP by both brush border and basolateral membranes. We also tested the ability of other protease inhibitors to prevent the degradation of VIP by basolateral membranes [26, 27]. Leupeptin (2 μg/ml), N-ethylmaleimide (1 mM), NH_4Cl (100 mM), soybean trypsin inhibitor (50 μg/ml), and phenylmethylsulfonyl fluoride (20 μg/ml) failed

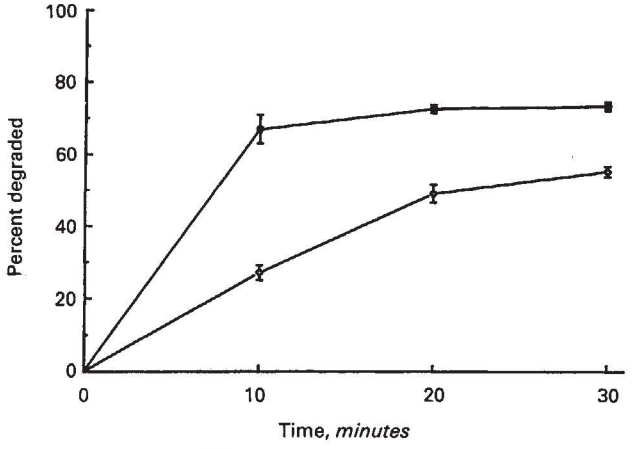

Fig. 1. *Degradation of ^{125}I-VIP by luminal (●) and basolateral membranes (◇). N = 3 to 4.*

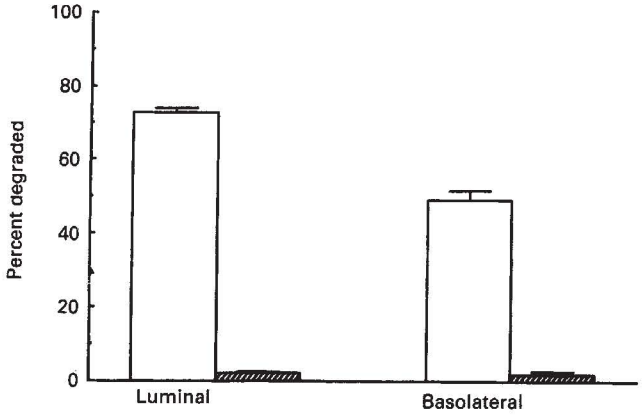

Fig. 2. *VIP degradation by luminal (left panel) and basolateral membranes (right panel) in the absence (open bars) and in the presence of 1% bacitracin (hatched bars). N = 8.*

to prevent the degradation of ^{125}I-VIP as compared to control. Therefore, in all subsequent experiments we utilized 1% bacitracin to prevent degradation of VIP.

Binding of ^{125}I-VIP by basolateral and brush border membranes

Figure 3 shows the displacement of ^{125}I-VIP bound to basolateral membranes by excess amounts of unlabeled VIP. It can be seen that unlabeled VIP displaced ^{125}I-VIP bound to basolateral membranes in a dose dependent fashion with 50% displacement at 2×10^{-7} M and maximal displacement at 10^{-6} M. Not shown is the fact that other hormones PTH, secretin and somatostatin failed or displaced minimally ^{125}I-VIP bound by basolateral membranes.

The data of VIP binding to basolateral membranes was analyzed by the saturation binding curve program defined by the equation $y = A^*X/(B + X)$, where X is the concentration of unlabeled VIP, y is the amount of specifically bound VIP, B is the dissociation constant (Kd) and A is the B_{max} [33]. This curve fitted well the data as evidenced by a correlation coefficient of 0.84 and a Kd of 2.2×10^{-7} M. Thus, the Kd determined from the displacement curve is close to Kd determined satura-

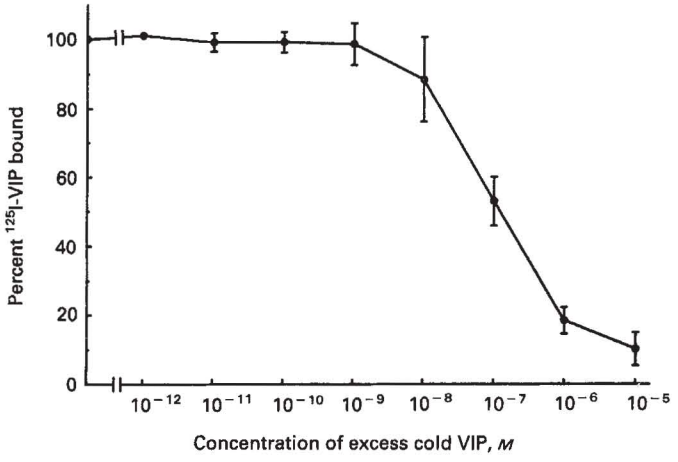

Fig. 3. *Displacement of ^{125}I-VIP bound to basolateral membranes by excess of unlabeled VIP. N = 3 to 10.*

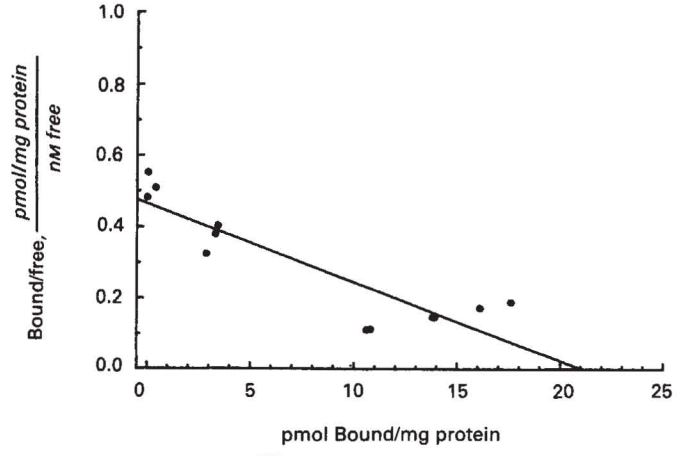

Fig. 4. *Scatchard plot of ^{125}I-VIP binding to basolateral membranes. N = 6.*

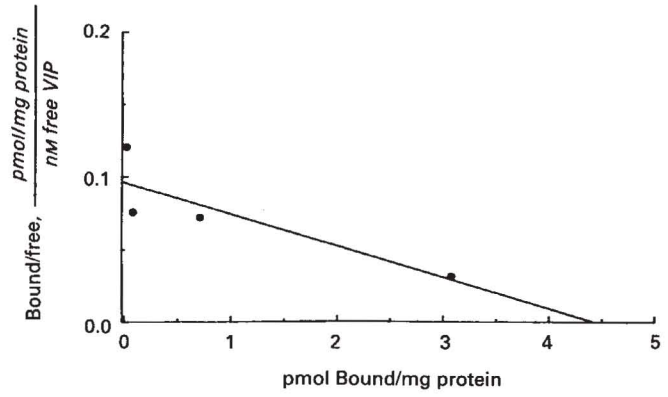

Fig. 5. *Scatchard plot of ^{125}I-VIP binding to luminal membranes. N = 4; r = 0.86.*

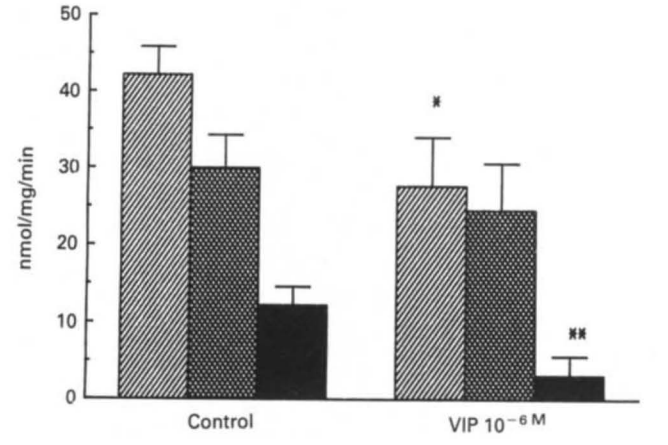

Fig. 6. *^{22}Na uptake at 1 min by acid-loaded proximal tubule suspension under control condition (left panel) and in presence of 10^{-6} M VIP (right panel).* Asterisks refer to comparison to control $*P < 0.005$, $**P < 0.025$. $N = 8$. Symbols are: (▨) total; (▩) + amiloride; (■) amiloride sensitive.

tion binding curve program. These data were also analyzed by Scatchard plot which is shown in Figure 4. These data are well fitted by linear regression with correlation of coefficient of 0.89, suggesting one class of receptor for VIP in the basolateral membranes with maximal number of binding of 22 pmol/mg protein and Kd of 0.5×10^{-7} M. Thus, the Kd determined from the Scatchard analysis is close to the apparent Kd determined from the saturation curve. Figure 5 shows the Scatchard plot of VIP binding to brush border membranes. Again, one single class of receptor for VIP is disclosed with B_{max} of 4.4 pmol/mg protein and Kd of 0.4×10^{-7} M. There was 8% cross contamination of the luminal membranes with basolateral membrane (**Methods**). This contamination of brush border membrane with basolateral membranes can account for 2.1 pmol/mg protein (48%) of VIP binding to brush border membranes.

Effect of VIP on tubule transport

Preliminary experiments indicated that ^{22}Na uptake increased with time and reached a peak level at one minute; for this reason all subsequent experiments were performed at one minute. Figure 6 shows ^{22}Na uptake at one minute in presence of 20 mM Na by isolated proximal tubules loaded with acid (pH 6.4) and suspended in buffer of pH 7.4 to generate a pH gradient. It is clear that there is ^{22}Na uptake which is inhibited approximately 30% by amiloride (10^{-3} M), indicating the presence of Na-H antiporter in the isolated proximal tubule suspension. Addition of 10^{-6} M VIP resulted in a significant 35% inhibition of total ^{22}Na uptake as compared to control tubules ($P < 0.005$). VIP inhibited the amiloride-sensitive ^{22}Na uptake by 75% at 10^{-6} M ($P < 0.025$). The amiloride-insensitive ^{22}Na uptake was not inhibited by VIP.

Figure 7 shows evidence of Na-dependent glucose transport in the isolated tubule suspension as evidenced by the fact that α-MGP uptake was higher in the presence of Na than K. α-MGP uptake peaked at one minute and reached an equilibrium at 60 minutes. The effect of VIP (10^{-5} M) in α-MGP uptake in the presence of Na or K was examined at 15 seconds, 1 minute, 5 minutes, and 60 minutes. At all time intervals, VIP failed to alter Na dependent α-MGP uptake (control 1.82 ± 0.25 vs. 1.60 ± 0.18 nmol/mg protein at 1 min, $N = 13$).

Table 1 shows Na-dependent ^{32}phosphate uptake in absence and in the presence of 10^{-5} M VIP at one minute. At times 15

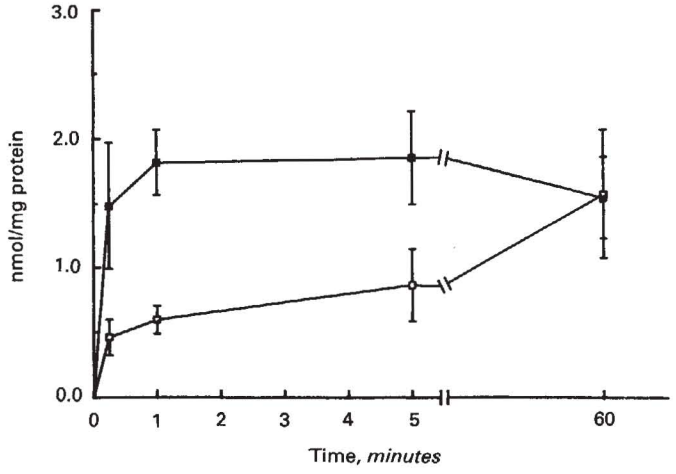

Fig. 7. *Methyl α-D ^{14}C-glucopyronoside uptake by proximal tubule suspension in presence of sodium (■) and in presence of potassium (□). *$P < 0.05$, $N = 13$.*

seconds, 30 seconds, 1 minute and 60 minutes there was stimulation of ^{32}phosphate uptake in presence of Na^+ as compared to K^+, indicating the presence of Na-dependent phosphate uptake. At this and all other time intervals, VIP failed to alter Na-dependent or independent phosphate uptake.

Effect of VIP on cyclic AMP generation by isolated proximal tubule

Figure 8 shows cyclic AMP before (basal) and after addition of different hormones. As expected PTH and forskolin stimulated, while AVP did not alter cyclic AMP levels. VIP (10^{-7} M), not shown up 10^{-5} M, failed to stimulate cyclic AMP production. In presence of IBMX, VIP also failed to stimulate cyclic AMP levels.

Discussion

Our study provides evidence for presence of specific receptors for VIP in highly-purified renal brush border and basolateral membranes. These membranes are capable of degrading VIP and studies on proximal tubule suspension strongly suggest that VIP inhibits the Na-H antiporter. Previous studies in man, in animals and in the isolated perfused kidney have suggested a role for VIP in renal function, but from these studies it is unclear whether the effect of VIP on renal function is due to the direct tubular effect or to the hemodynamic effects elicited by VIP [18–21]. Previous studies have attempted to determine binding and degradation of VIP in plasma membranes of the renal cortex and in kidney slices [34, 35]. Griffiths and Simmons [34] demonstrated the presence of specific binding and degradation of VIP in plasma membranes from the cortex and renal medulla. They found that cortical membranes were capable of 30% degradation of VIP with maximal binding sites of 0.219 nmol/mg protein and half maximal binding of 3.3 μM. More recently Griffiths and Simmons [35] reported that the binding of VIP to microsomes of the feline renal cortex had a Kd of 8.4 μM for the high affinity receptor, which is very close to the results found in the present study. Magistretti et al [36] found half maximal binding of 10 μM utilizing autoradiography of kidney slices. In both of these studies it is impossible to determine the contribution of the brush border versus the basolateral membranes in binding and degradation of VIP. In the present study we characterized binding and degradation of VIP by highly-purified brush border and basolateral renal cortical membranes and thus could determine the relative contribution of these membranes to VIP binding and degradation. Our studies clearly demonstrate a much lower Kd and higher B_{max} as compared to previous studies. This difference is undoubtedly accounted for by the use of highly-purified renal cortical membranes. We found that degradation of VIP was higher in the brush border membranes than in basolateral membranes, while in the latter the maximal number of binding sites was six times greater than in the brush border membranes. As discussed in the results, 48% of VIP binding to brush border membranes can be accounted for by cross contamination of these membranes with basolateral membranes. This pattern of binding and degradation are in agreement with studies of insulin degradation and binding in that the brush border membranes were capable of more degradation while having lower number of maximal binding sites for this hormone than the basolateral membranes [26]. As compared to studies performed in purified intestinal membranes [1] our studies show a similar Kd and number of maximal binding sites.

Having demonstrated the presence of specific binding site for VIP in renal cortical membranes, we attempted to uncover an effect of VIP on transport by studying the effect of this hormone on isolated proximal tubular suspension. Proximal tubule suspension contains a Na-H antiporter, Na-glucose cotransporter and Na-phosphate cotransporter; these systems have been well characterized in vitro [28–30]. In the present study, the rate of ^{22}Na uptake, (in presence of 20 mM Na), in acid-loaded proximal tubules was comparable to that recently reported for rat proximal tubules but lower than that measured in rabbit proximal tubules [29, 37]. In addition, amiloride inhibited ^{22}Na uptake by 30%, a value close to the 40% inhibition observed with ethylisopropylamiloride, a more potent inhibitor of the Na-H antiporter [37]. VIP caused a significant inhibition of ^{22}Na uptake in acid-loaded proximal tubules and affected only the amiloride sensitive component of the ^{22}Na uptake. At a lower concentration of Na (1 mM) VIP also inhibited the amiloride sensitive component of the Na-H antiporter (data not shown).

Since VIP has been reported to have a weak inhibitory effect on Na-dependent phosphate transport in the opossum kidney cells [38], we examined the effect of VIP on Na-dependent phosphate transport in proximal tubules. As reported by other investigators [30], we found that in the presence of Na as compared to K, phosphate uptake was higher indicating the presence of Na-phosphate cotransporter. VIP, however, failed to inhibit Na-dependent phosphate transport. Likewise, VIP also failed to inhibit Na-dependent glucose transport. These results suggest that the effect of VIP is relatively specific for the Na-H antiporter in the proximal tubule. The results of the present study suggest that VIP may inhibit acidification not only by stimulating HCO_3 secretion [17] but also by inhibiting the Na-H antiporter.

Cyclic AMP is thought to be the mediator of VIP in many tissues [39–41]. Because cyclic AMP is well known to inhibit the Na-H antiporter and phosphate transport we measured the effect of VIP on cyclic AMP generation by proximal tubule suspension [42]. VIP caused minimal or no increase in cyclic

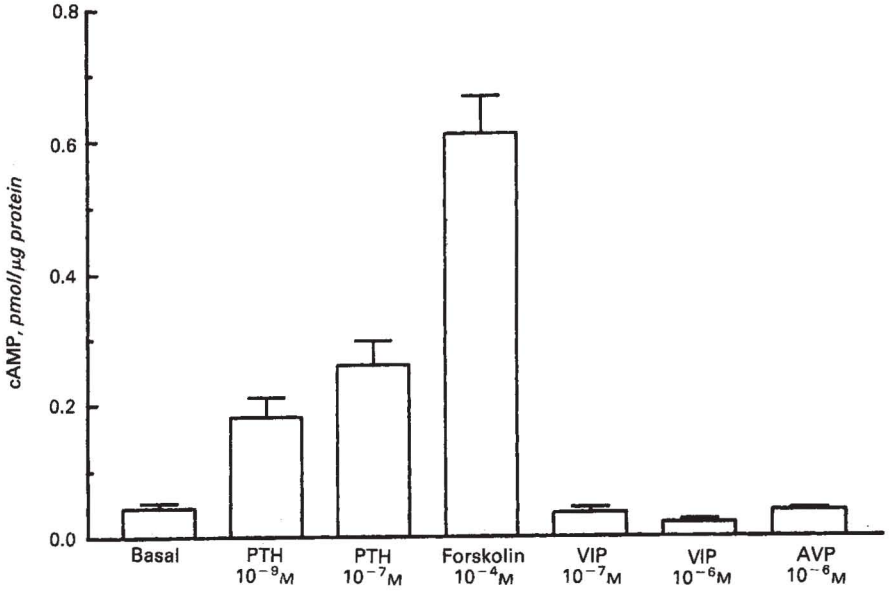

Fig. 8. *Cyclic AMP generation by proximal tubular suspension under basal conditions and in presence of different hormones. N = 10.*

Table 1. Na-dependent ^{32}phosphate uptake (n:20; nmol/mg protein/60 sec)

	Control	*P*	VIP (10^{-5} M)
Sodium	5.8 ± 0.98	NS	5.70 ± 1.03
	$P < 0.05$		$P < 0.001$
Potassium	1.36 ± 0.22	NS	1.48 ± 0.26

AMP generation while PTH and forskolin caused the expected increase in cyclic AMP. These findings indicate that preparation was adequate to disclose an effect of VIP on cyclic AMP generation. Griffiths and Simmons [41] initially reported that VIP increased adenylate cyclase activity by rabbit renal basolateral membranes 1.6-fold. However, in a subsequent study, these investigators measured the effect of VIP on adenylate cyclase activity in isolated tubule segments of the rabbit kidney [42]. They found that VIP stimulated adenylate cyclase activity in the distal convoluted tubule and in the medullary collecting duct, but failed to stimulate the activity of this enzyme in the proximal tubule. Thus, our results are in agreement with the latest results of Griffiths and Simmons et al [35, 43].

In conclusion, the results of the present study demonstrate the presence of specific receptors for VIP in the proximal tubule and suggest that VIP inhibits the Na-H antiporter by a mechanism other than cyclic AMP. Further studies are necessary to elucidate the mechanism whereby VIP exerts its tubular effect.

Acknowledgments

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